ATTACHMENTS

B. Ferrandes et al. "Metabolism of Progabide in Four Animal Species and in Man" in *L.E.R.S.* Vol. 3, pages 217-229.

REMARKS

With this amendment, claims 1-3, 5-13, 15-18 and 26 are pending in the application. Claims 1, 11, 18 and 26 are the only claims in independent form. Claims 4, 14 and 27-39 are hereby canceled.

Applicant affirms that the subject matter of the various claims was commonly owned at the time of the invention.

Remarks Directed to Rejection of Claims 1-3, 5-13 and 15-18 under 35 U.S.C. §112

Claims 1-3, 5-13 and 15-18 stand rejected under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement due to amendment of the claims to include the proviso that the compound [α-(chloro-4'phenyl) fluoro-5' hydroxyl-2-benzylidene-amino]-4 butyramide is not a compound included in a claimed invention. Applicant hereby amends claims 1, 11 and 18 to delete reference to this compound and therefore submits that the rejection is moot. Applicant respectfully requests that this rejection be withdrawn.

Remarks Directed to Rejection of Claims 1-3, 5-13, 15-18 and 26 under 35 U.S.C. §103(a) over Aebischer et al.

Currently, claims 1-3, 5-13, 15-18 and 26 stand rejected under 35 U.S.C. §103(a) over Aebischer et al. (U.S. Patent 5,474,547). Aebischer et al. is cited for teaching the alleviation of movement disorders associated with Parkinson's and Huntington's diseases through the administration of GABA, GABA agonists and GABA potentiators by implantation of devices. (Paper No. 01122004, page 3, last paragraph, maintained Paper No. 08182005, page 2, last

paragraph). The Examiner asserts that "broad claim language" of claims directed to "an effective amount of the compound gamma-aminobutyramide, analogs, substituted forms, derivatives, the pharmaceutically acceptable salts, esters, amides and prodrugs thereof" includes "the GABA, GABA agonists and GABA potentiators of Aebischer et al." Claims 1, 11, and 18 are hereby amended to clarify that methods are provided according to the present invention which include administration of the compound gamma-aminobutyramide or a pharmaceutically acceptable salt thereof.

> In order "[t]o establish a prima facie case of obviousness ... the prior art reference (or references when combined) must teach or suggest all the claim limitations." (MPEP 2143)

The pending claims teach the use of gamma-aminobutyramide (GABAmide) in certain disorders including spastic disorders, convulsions, epilepsy, idiopathic dystonia and torsional dystonia.

Applicant finds no apparent description of use of gamma-aminobutyramide in the Aebischer et al. reference. Thus, the Aebischer et al. reference does not appear to teach or suggest all the limitations of the present claims. It is therefore submitted that no *prima facie* case of obviousness is established and it is respectfully requested that the rejection as to claims 1-3, 5-13, 15-18 and 26 under 35 U.S.C. §103(a) over Aebischer et al. be withdrawn.

Remarks Directed to Rejection of Claims 1-3, 5-13, 15-18 and 26 under 35 U.S.C. §103(a) over Bergmann

Currently, claims 1-3, 5-13, 15-18 and 26 stand rejected under 35 U.S.C. §103(a) over Bergmann (*Clinical Neuropharmacology* Vol. 8, pages 13-26). Bergmann is cited as teaching "Progabide is metabolized to α chloro-4'phenyl fluoro-5 hydroxy-2-benzylidene amino 4 butanoate sodium and then to GABAmide." (Paper No. 08182005, page 5, second paragraph).

> In order "[t]o establish a prima facie case of obviousness ... there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference ..." (MPEP 2143)

The pending claims teach the use of gamma-aminobutyramide (GABAmide) in certain disorders including spastic disorders, convulsions, epilepsy, idiopathic dystonia and torsional dystonia.

In contrast to the present claims, the Bergmann reference does not appear to teach or suggest the administration of gamma-aminobutyramide in a method to treat the cited disorders. Instead, Bergmann teaches the use of progabide and describes several metabolites of progabide, including the corresponding acid ([α chloro-4'phenyl fluoro-5 hydroxy-2-benzylidene amino]-4-butanoate sodium, gamma-aminobutyramide, and GABA. (Bergmann, page 14).

As the present specification makes clear, "dissolving PROGABIDE in a solvent" generates "gamma-aminobutyramide and an insoluble ketone." (Page 11, lines 13-14). Administration of progabide results in the insoluble 4-chlorophenyl-5-fluoro-2-hydroxyphenylmethanone ketone in a treated individual, an undesired compound as a metabolite. The statements in the specification are supported by B. Ferrandes et al., *L.E.R.S.* Vol. 3, pages 217-229, a copy of which is appended to this amendment. The Bergmann reference does not appear to recognize this undesired product of progabide administration, and further, does not teach or suggest the administration of gamma-aminobutyramide to avoid this problem. In addition, Bergman does not appear to teach or suggest the administration of gamma-aminobutyramide as advantageous due to the fact that "[t]his compound is significantly more stable and has a longer half-life than PROGABIDE ..." (present specification page 11, lines 16-17). The fact that the Bergmann reference makes it clear that Bergmann knew about gamma-aminobutyramide but describes the uses and advantages of progabide emphasizes the nonobviousness of the methods of the present invention directed to gamma-aminobutyramide administration.

Thus, it is submitted that the Bergmann reference does not provide suggestion or motivation to modify the reference to use gamma-aminobutyramide as detailed in the present application. Rather, Bergmann does not teach problems associated with administration of progabide or advantages to administration of gamma-aminobutyramide.

The Examiner describes the instant application as containing dependent claims that differ from the Bergmann reference in describing intrathecal administration, intraventricular administration, administration by an implantable pump and administration by a spinal catheter. (Paper No. 08182005, page 5, second paragraph). Bergmann is described as teaching that "gastric-resistant formulations of progabide have been shown to result in incomplete absorption and lower serum levels." (Paper No. 08182005, page 5, second paragraph). It is asserted that "[s]ince the gastric-resistant formulations result in incomplete absorption, it would have been obvious to administer the compound by parenteral means." (Paper No. 08182005, page 5, second paragraph).

Applicant notes that the Bergmann reference describes "varying formulations of progabide" and describes the problem of incomplete absorption and lower, fluctuating serum levels as relating to only one type of formulation, the gastric-resistant formulation. Bergmann goes on to describe a micronized tablet formulation which "yields constant serum levels, better absorption and a somewhat shorter half-life ..." in contrast to the gastric-resistant formulation. (Bergmann, pages 14-15). Thus, Bergmann does not appear to teach or suggest limitations of oral formulations or systemic administration generally, limiting the description of a problem to a specific oral formulation only.

Thus, it is submitted that the Bergmann reference does not provide suggestion or motivation to modify the reference to administer gamma-aminobutyramide by intrathecal administration, intraventricular administration, by an implantable pump or by a spinal catheter as

detailed in the present application and as such represents a separate basis for allowability of the pending dependent claims.

It is therefore submitted that no *prima facie* case of obviousness is established and it is respectfully requested that the rejection as to claims 1-3, 5-13, 15-18 and 26 under 35 U.S.C. §103(a) over Bergmann be withdrawn.

Summary

Claims 1-3, 5-13, 15-18 and 26 are pending in the present application. Claims 1, 11, 18 and 26 are the only claims in independent form. Claims 4, 14 and 27-39 have been canceled and claims 1, 11 and 18 have been amended. Applicant submits that the present claims are believed to be in condition for allowance. Therefore, allowance of the pending claims and the passing of this application to issuance are solicited. Should the Examiner find to the contrary or have suggestions as to how to form of a pending claim may be improved, she is respectfully requested to contact the undersigned attorney to resolve any remaining issues.

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CERTIFICATE UNDER 37 CFR 1.8(a)

I hereby certify that this correspondence is being forwarded to the United States Patent Office via facsimile (571-273-8300) on November 23, 2005.

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Metabolism of Progabide in Four Animal Species and in Man

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The comparison of metabolic patterns in various animal species and man is an essential step during the development of a new drug.

The study of the metabolic degradation of a given molecule not only is of primary importance to validate the choice of an adequate animal species for toxicological studies but also permits a better understanding of the pharmacological profile of the new molecule and may help to shed some light on toxic phenomena observed during toxicological studies in animals and long-term administration to man. The identification of active metabolites and the definition of their pharmacokinetic profiles may lead to an improved understanding of the duration of action of a new drug and of additional effects not necessarily linked to the primary mechanism of action of the parent drug. Identification of highly reactive intermediates may be useful for a better definition of the mechanisms underlying possible toxic reactions in target organs as well.

With these prospective goals, the metabolism of progabide was investigated in four animal species (mouse, hamster, rat, and baboon) and in man. We report here the results of these studies together with data on the kinetics of progabide and its major active metabolite.

MATERIAL AND METHODS

Radioactive Compounds

Balance and metabolic studies were conducted in man and animals with ^{14}C-progabide labelled in the benzophenone moiety (progabide B).

Additional studies were performed, in animals only, with ^{14}C-progabide labelled in the GABAmide side chain (progabide G). These compounds (Fig. 1) were obtained with a specific radioactivity of 43.6 mCi/mmole (progabide B) and 58.1 mCi/mmole (progabide G) from the radiochemical unit of L.E.R.S. (Bagneux, France).

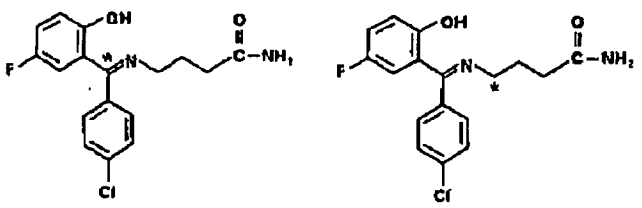

FIG. 1. Radiolabelled progabide used in the metabolic and kinetic studies.

Animal Studies

Balance Studies

a first set of experiments, mice, hamsters, rats, and baboons were dosed enously or orally with 20 mg/kg of ^{14}C-progabide B corresponding to 100 g (mice, hamsters, rats) and 20 μCi/kg (baboons). In additional experi-, rats and baboons were dosed by the same routes of administration with g/kg of ^{14}C-progabide G. During these studies, urine and feces were col- separately at 0 °C for each animal until complete recovery of the admin- dose. The elimination of $^{14}CO_2$ in the expired air was also measured in id baboons after administration of progabide G together with the resid- dioactivity present in the carcasses of the rats.

Metabolism

tative study

metabolites were isolated from bile or urine of rats dosed intravenously lly with 20 mg/kg of progabide B according to the diagram presented in . The structure of these compounds was elucidated by mass spectrometry. ding on the compounds, the analysis was performed after direct intro- n of the metabolites or gas chromatography–mass spectrometry MS) of their trimethylsilyl derivatives using a Girdel 32 gas chromato- coupled with a Ribermag R 10-10/SIDAR mass spectrometer. The cor- ding authentic compounds were then synthesised for confirmation of the sed structures.

antitative study

The relative amounts of metabolites in biological samples were estimated in ouse, hamster, rat, and baboon after intravenous or oral administration of 20

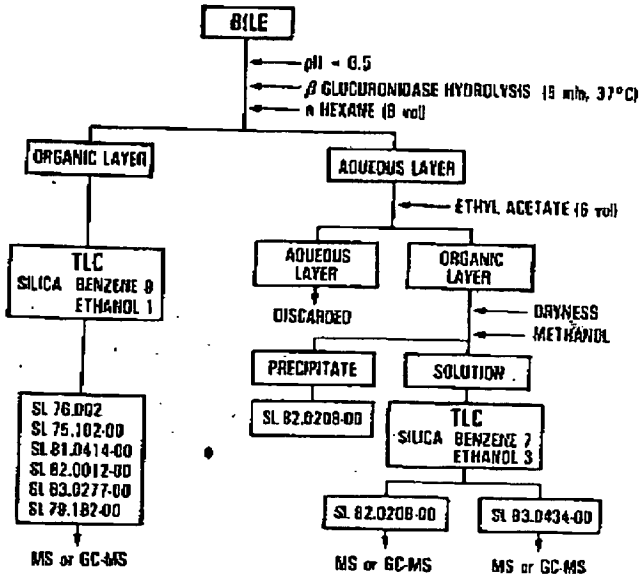

FIG. 2. Separation and purification of radioactive metabolites from bile of rats dosed with ^{14}C-progabide.

mg/kg of progabide B. The metabolites present in the hydrolysed samples (β-glucuronidase) were separated by high performance liquid chromatography (HPLC), and radioactivity was measured in the fractions by liquid scintillation.

Autoradiography

Autoradiography was performed in rats dosed intravenously or orally with progabide in the B (20 mg/kg) or G (1 mg/kg) form. At various time intervals from 15 min to 24 hr the animals were sacrificed, and sections were prepared according to conventional methods (3).

Plasma and Tissue Kinetics

The plasma kinetics of progabide and its acid metabolite were estimated in the mouse, hamster, rat, and baboon after intravenous or oral administration of 20 mg/kg of progabide B (1).

The plasma levels of these compounds were measured by HPLC with electrochemical detection according to the method described by Yonekawa et al. (4). In the rat, the brain regional distributions of progabide and of two of its metabolites were also studied. The brain was dissected into nine structures ac-

ling to Glowinski and Iversen (2), and the determinations of the com-
nds were performed by HPLC with UV or electrochemical detection.

Human Studies

Balance and Metabolic Studies

x hundred milligrams of progabide B corresponding to 50 μCi were sus-
ied in 0.5% hydroxypropylmethylcellulose and administered orally to three
thy volunteers weighing, respectively, 89, 78, and 73 kg. Urine and feces
: collected separately at intervals from 0 to 96 hr and analysed for total ra-
ctivity. Urine samples were also used after β-glucuronidase hydrolysis for
dentification and quantification of the metabolites. Identification was per-
ed by gas chromatography–mass spectrometry (GC–MS) on the trimeth-
yl derivatives of the metabolites previously isolated by HPLC. The
atification of these compounds was performed by HPLC with measurement
adioactivity (liquid scintillation) in collected fractions.

Pharmacokinetic Profile in Healthy Volunteers

harmacokinetic studies in healthy volunteers were conducted after oral ad-
istration of 600 mg of nonradioactive progabide to 76 subjects aged from 18
) years (mean 25.9 years). Blood was sampled up to 36 hr whenever possi-
or the determination of unchanged progabide and its main metabolite.

RESULTS

Metabolism of Progabide in Animals and Man

imination of Radioactivity in the Mouse, Rat, Hamster, Baboon, and Man

fter intravenous administration of progabide B, total radioactivity was ab-
t completely eliminated in 48 hr in the four animal species. The pattern of
ination differs from one species to another as indicated in Table 1. The
route of elimination appears to be predominant in the rat (76.9 ± 2.9% of
dministered dose) but less important or minor in the other species (for ex-
le 10.4 ± 1.0% in hamster).
ne experiments conducted in rat, hamster, and baboon demonstrated that
iliary elimination of radioactivity was slightly greater than the fecal and
an enterohepatic circulation of radioactivity may be suspected in these
species.
After oral administration of progabide B (Table 2), similar results were ob-
served in the mouse, hamster, and baboon, whereas rat urinary elimination of
radioactivity is significantly higher than after intravenous administration (re-

TABLE 1. *Balance study following intravenous administration of ^{14}C-progabide (B form) to the mouse, hamster, rat, and baboon*

Species	Dose (mg/kg)	Radioactivity (μCi/kg)	Duration (hr)	Urine[a]	Feces[a]	Urine plus feces[a]
Mouse (n = 9)	20	100	48	48.1 ± 2.3	45.1 ± 2.0	91.2 ± 4.2
Hamster (n = 4)	20	100	120	82.9 ± 0.9	10.4 ± 1.0	94.0 ± 1.6[b]
Rat (n = 3)	20	100	72	18.6 ± 2.3	78.9 ± 2.9	95.6 ± 3.7
Baboon 1	20	20	120	66.1	16.1	96.3[b]
Baboon 2	20	20	120	83.4	16.5	98.4[b]

[a] The results are expressed as a cumulative percentage of the administered dose (individual values or mean ± SE).
[b] Including radioactivity present in the cage-washing liquids.

TABLE 2. *Balance study following oral administration of ^{14}C-progabide (B form) to the mouse, hamster, rat, baboon, and man*

Species	Dose (mg/kg)	Radioactivity (μCi/kg)	Duration (hr)	Urine[a]	Feces[a]	Urine plus feces[a]
Mouse (n = 9)	20	100	48	57.8 ± 0.6	36.1 ± 0.7	93.9 ± 1.1
Hamster (n = 4)	20	100	120	87.7 ± 3.1	11.9 ± 2.4	99.8 ± 0.9[b]
Rat (n = 3)	20	100	72	49.3 ± 5.3	48.4 ± 5.0	97.8 ± 0.3
Baboon 1	20	20	120	72.3	4.9	89.1[b]
Baboon 2	20	20	120	46.7	10.9	84.7[b]
Man 1	6.7	0.56	96	64.7	8.9	73.5
Man 2	7.7	0.64	96	56.9	7.2	64.1
Man 3	8.2	0.68	96	78.3	6.5	84.9

[a] The results are expressed as a cumulative percentage of the administered dose (individual values or mean ± SE).
[b] Including radioactivity present in the cage-washing liquids.

pectively, 49.3 ± 5.3% and 18.6 ± 2.3%). In man, the radioactivity associated
ith progabide **B** is mainly excreted in urine, with 57 to 79% of the dose recov-
red in urine during the first 96 hr following oral administration of 600 mg of
rogabide; 6.5 to 8.9% of the dose was found in the feces. The incomplete total
adioactivity recovery observed in man, 64 to 85%, may be explained by the
act that the fecal excretion was not complete during the sampling time.

Additional experiments conducted in rat and baboon with progabide G (Table 3) demonstrated a very different pattern of elimination of radioactivity. Urinary and fecal routes only accounted for part of the administered dose, and ignificant amounts of $^{14}CO_2$ were found in the breath in both species.

Comparison of the results obtained with progabide B and G demonstrates hat a cleavage of the imine bond occurs during the metabolism of progabide, nce the elimination profile of radioactivity differs according to the position of ^{14}C in the labelled molecule. GABA and GABAmide resulting from this cleav-ge and liberated in the organism are then incorporated into the intermediate netabolism with subsequent release of $^{14}CO_2$.

Metabolic Pathways of Progabide in Animals

In animals, progabide is metabolized according to four major routes of bio-ransformation. The combination of these routes leads to 10 metabolites, as in-licated in Fig. 3.

1. Hydrolysis of the amide group on the GABAmide side chain leads to acid erivatives (SL 75.102–00, SL 83.0434–00, and SL 82.0054–00).
2. Hydrolysis of the imine bond leads to benzophenone derivatives (SL 9.182–00, SL 83.0277–00, and SL 81.0414–00) and to GABA and GABAmide.
3. Hydroxylation on C-3 of the 5-fluoro-2-hydroxyphenyl ring leads to *ortho*-dihydroxy compounds (SL 82.0208–00, SL 83.0434–00, and SL 3.0277–00).
4. Hydroxylation on C-5 of the 5-fluoro-2-hydroxyphenyl ring leads to *para*-dihydroxy compounds (SL 82.0012–00, SL 82.0054–00, and SL 1.0414–00).

These metabolites are eliminated mainly as their glucuroconjugate deriva-ives, but SL 79.182–00 was also observed in rat and baboon urine as a sulfo-onjugate (SL 83.0625–00). The relative amounts of these metabolites in urine r bile are presented in Table 4.

Independent of the route of administration, unchanged progabide is only liminated in trace amounts. After intravenous administration of progabide, he *ortho*-dihydroxy metabolites account for 47, 43, and 29% of the adminis-ered dose in the hamster, rat, and baboon, respectively, whereas after oral treatment these metabolites are fairly less important (30, 8, and 8% in the same three species). In contrast, SL 79.182–00 resulting from the hydrolysis of the imine bond is more abundant in the glucuro- or sulfoconjugated form after oral

TABLE 3. *Balance study following intravenous administration of ^{14}C-progabide (G form) to the rat and baboon*

Species	Dose (mg/kg)	Radioactivity (μCi/kg)	Urine[a]	Feces[a]	Expired $^{14}CO_2$[a]	Residual radioactivity in carcass
Rat ($n = 3$)	1	100	48.0 ± 3.1 (*t* = 72 hr)	10.2 ± 0.5 (*t* = 72 hr)	25.1 ± 3.7[b] (*t* = 24 hr)	17 (*t* = 72 hr)
Baboon 1 (M)	20	20	14.2 (*t* = 120 hr)	3.4 (*t* = 120 hr)	32.8 (*t* = 24 hr)	
Baboon 2 (F)	20	20	26.9 (*t* = 120 hr)	5.9 (*t* = 120 hr)	26.8 (*t* = 24 hr)	

[a] The results are expressed as a cumulative percentage of the administered dose (individual values or mean ± SE).
[b] $^{14}CO_2$ was collected in a separate experiment.

TABLE 4. *Percentages of elimination of progabide and metabolites in the mouse, hamster, rat, baboon, and man*[a]

Compound	Mice[b]		Hamster[a]		Rat[a]		Baboon[a]		Man[a]
	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.	p.o.
SL 76.002	<1	<1	<1	<1	<1	<1	<1	<1	<1
SL 75.102-00	<1	<1	<1	<1	<1	1	<1	<1	<1
SL 79.182-00[d]	10	25	15	39	31	67	55	43	
SL 82.0012-00	3	3	6	4	3	2	3	1	
SL 82.0054-00	3	3	6	3	2	4	1	1	
SL 81.0414-00	13	12	5	3	1	2	1	<1	2
SL 82.0208-00 SL 83.0434-00 SL 83.0277-00	11	9	48	30	43	6	28	6	20
Total	40	51	81	80	86	82	73	65	63

[a] The results are expressed as a percentage of the administered dose.
[b] Urinary data.
[c] Sum of urinary and biliary data.
[d] Sum of glucuroconjugate and sulfoconjugate (SL 83.0625-00).

FIG. 3. Metabolic pathways of progabide in the mouse, hamster, rat, baboon, and man.

administration of progabide than after intravenous treatment (respectively, 38 and 15% in the hamster, 67 and 31% in the rat, and 55 and 32% in the baboon). This indicates that the cleavage of the imine bond is an important route of biotransformation, occurring in the animal species during the absorption of the compound.

Except in the hamster, and in the mouse, where the corresponding metabolites account in urine for approximately 18% of the administered dose, C-5 hydroxylation appears to be a minor route of biotransformation.

Metabolic Pathways of Progabide in Man

The metabolic pathways of progabide in man are qualitatively and quantitatively similar to those observed in the animal species. The GABAmide side chain of progabide is deamidated to yield an active carboxylic acid SL 75.102–00. Both progabide and SL 75.102–00 are present in plasma in concentrations of the same order of magnitude.

The major metabolites found in urine result from the hydrolysis of the imine

ond and account for 55.6 to 75% of the administered dose. Among those metabolites, SL 79.182–00, formed by hydrolysis of progabide, represents 3.0 ± 4.7% of the dose. Two hydroxylated derivatives are found in lower mounts: SL 83.0277–00 (19.6 ± 2.0% of the dose) and SL 83.0414–00 1.9 ± 0.2%).

Neither progabide nor SL 75.102–00 is found in urine with our analytical ethod, which has a limit of detection of 10 ng/ml for both compounds. The ydroxy metabolite of progabide, SL 82.0208–00, is excreted in small amounts 1.2% of the dose). This metabolite yields SL 83.0277–00 on hydrolysis.

It should be pointed out that the percentages given above for the urinary excretion of the metabolites depend on the pharmaceutical form. In particular, ie importance of SL 79.182–00 may be overestimated since the formulation dministered in this study could not be micronised, and it is known from previous studies that micronisation minimises the hydrolysis of the imine bond in ie gastrointestinal tract. In conclusion, these results observed in man are in ood agreement with those described above for the animal species.

Kinetics of Progabide and Metabolites

SL 75.102–00, the pharmacologically active acid derivative of progabide, as followed in the four animal species and in man. A comparison of the kinetics of this metabolite with that of the unchanged compound demonstrates the onger apparent elimination half-life of SL 75.102–00 in all species. In mouse nd rat, this metabolite is found at greater levels than progabide, but in hamster, baboon, and man, this metabolite is less abundant in plasma than the nother drug (Table 5) after a single dose.

TABLE 6. *Comparison of pharmacokinetic parameters calculated from plasma concentrations of progabide and SL 75.102-00 after oral administration of progabide to four animal species (20 mg/kg) or man (600 mg)*

Species	$t_{1/2}$ progabide (hr)	$t_{1/2}$ SL 75.102-00 (hr)	AUC SL 75.102-00/AUC progabide
Mouse	0.2	0.6	3.6
Hamster	0.5	1.4	0.9[a]
Rat	0.4	3.6	5.1
Baboon	0.6	—[b]	—[b]
Man	3.1	8.6	0.8

[a] Value calculated after 200 mg/kg.
[b] SL 75.102-00 was detected in trace amounts in baboon.

Distribution Studies in the Rat

Autoradiography

The autoradiographic studies conducted in rats demonstrate the overall distribution and elimination of progabide together with its radioactive metabolites. At short times after intravenous administration of progabide B, radioactivity is homogeneously distributed throughout the organism with good penetration of radioactivity into the brain (Fig. 4). It is interesting to note that at very short times after intravenous administration of progabide, radioactivity is preferentially located in the grey matter and then from 0.25 to 2 hr the inverse phenomenon is observed, with persistence of radioactivity in the white matter. Elimination of radioactivity occurred quite rapidly, and no accumulation of radioactive material in tissue was observed.

After administration of progabide G, a completely different picture is observed with persistence of radioactivity in tissues 24 hr after administration of the compound. This is related to the release of GABA or GABAmide resulting from the cleavage of the imine bond of progabide and related metabolites and their incorporation into the intermediate metabolism.

Brain Regional Distribution of Progabide and Two Metabolites

Additional studies conducted in rat demonstrated the presence of progabide and SL 75.102–00 (active acid metabolite) in the brain. It is of interest to note that brain radioactivity after administration of progabide B can be quantitatively explained by the presence of progabide, SL 75.102–00, and SL 79.182–00. In contrast, after administration of progabide G, the sum of proga-

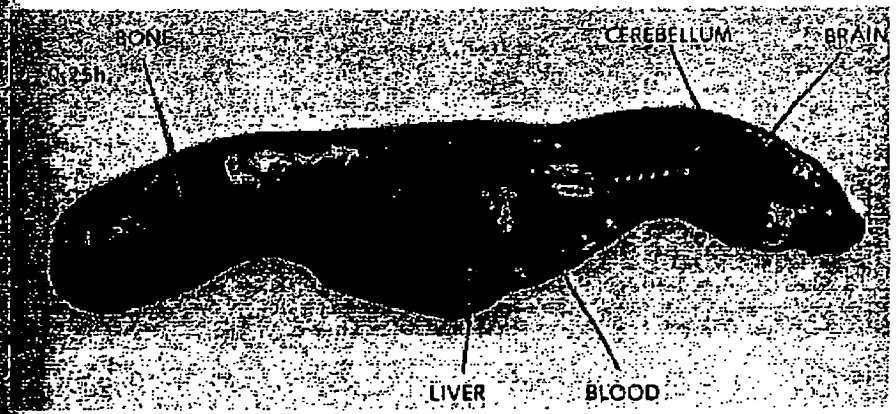

FIG. 4. Whole-body autoradiography of a male rat 15 min after intravenous administration of 20 mg/kg of ^{14}C-progabide B (100 μCi/kg).

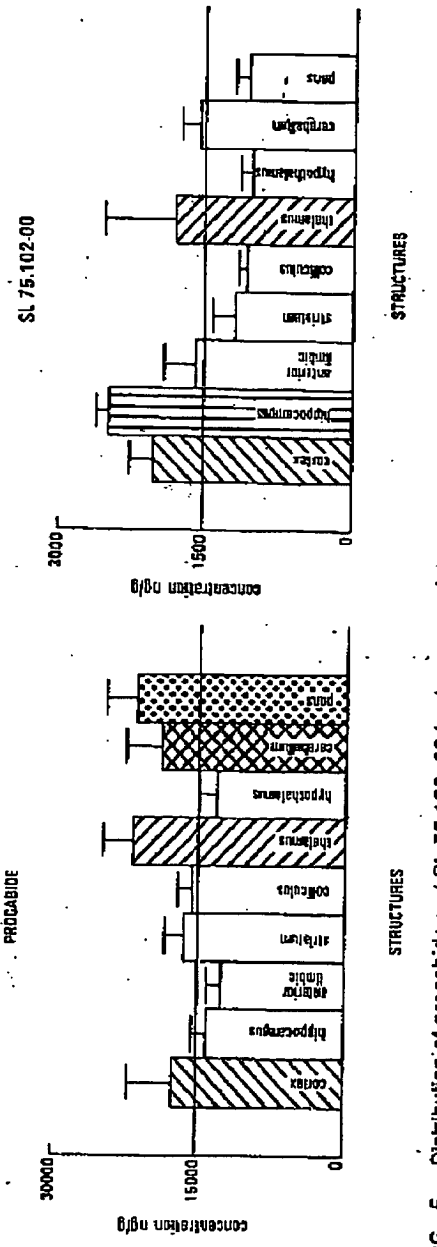

FIG. 5. Distribution of progabide and SL 75.102–00 in nine areas of the brain 0.25 hr after intravenous administration of 20 mg/kg of progabide to the rat.

bide and SL 75.102–00 does not account for the brain radioactivity, demonstrating the presence of GABA or related compounds in the brain.

Finally, the regional distribution of progabide and SL 75.102–00 in discrete areas of the brain demonstrated a preferential localisation together with a different pattern of distribution of these two compounds in the central nervous system (Fig. 5). Progabide appeared to be more abundant in the cortex, thalamus, cerebellum, and pons, whereas SL 75.102–00 was more densely distributed in cortex, hippocampus, and thalamus. This distribution was observed from 5 min and persisted up to 2 hr after the administration of progabide.

CONCLUSION

The description of the metabolic fate of progabide presented in this chapter indicates that the compound is metabolised in the mouse, hamster, rat, baboon, and man via the same pathways: deamidation, cleavage of the imine bond, and hydroxylation of the benzophenone moiety (C-3 or C-5 position).

The possible combinations of these reactions may lead to 10 metabolites, which are excreted rapidly in urine and bile, mainly as their conjugates. As usual, biliary elimination appeared to be predominant in the rat but fairly less important in other species (especially in hamster and man). In all species, the importance of the hydrolysis of the imine bond was demonstrated. Kinetic experiments performed in all of these species showed the presence of progabide and of an active metabolite (SL 75.102–00) in blood. Moreover, studies performed in rat made it possible to observe these two active molecules in the brain of the animals in areas known to be involved in the genesis and the spreading of seizure activity. This suggets that in addition to the intrinsic activity of progabide, SL 75.102–00 may participate to some extent in the overall activity of the compound.

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REFERENCES

1. Ferrandes, B., Durand, A., Fraisse-André, J., and Morselli, P.L. (1984): Metabolic studies of progabide (SL 76.002) in the rat. In: *Metabolism of Antiepileptic Drugs*, edited by R. Levy et al., pp. 183–191. Raven Press, New York.
2. Glowinsky, J., and Iversen, L.L. (1966): Regional studies of catecholamines in the rat brain. *J. Neurochem.*, 13:655–669.
3. Waddell, W.J. (1971): Autoradiography in drug disposition studies. In: *Fundamentals of Drug Metabolism and Drug Disposition*, edited by B.N. La Du, H.G. Mandel, and E.L. Way, pp. 505–514. Williams & Wilkins, Baltimore.
4. Yonekawa, W., Kupferberg, H.J., and Lambert, T. (1983): Measurement of progabide and its deaminated metabolite in plasma by high performance liquid chromatography and electrochemical detection. *J. Chromatogr.*, 276:103–110.